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FastFung: A novel medium for the culture and isolation of fastidious fungal species from clinical samples

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17 **Abstract**

18 We developed a novel culture medium, referred to FastFung medium as suitable for the culture
19 of clinical fungi, including fastidious ones, for both research and diagnostic studies. It is based
20 on Schædler agar supplemented with many essential components for the growth of fastidious
21 fungi. It also contains selective antibacterial agents for the inhibition of contaminant bacteria
22 growth. In this preliminary study, the FastFung medium was compared to the gold standard
23 Sabouraud medium for 98 fungal and 20 bacterial strains.

24 The fungal strain positive culture rate was 100% vs. 95% and the bacterial strain inhibition was
25 100% vs. 20%, for the FastFung and Sabouraud media, respectively. When compared to the
26 Sabouraud medium on 120 clinical samples, the FastFung medium displayed both a higher
27 fungal colonies count, and a lower culture contamination rate. Storage at 4°C for 4 weeks did not
28 alter the FastFung culture medium performances for the six isolates of *Candida*, *Cryptococcus*,
29 and *Penicillium* tested.

30 These encouraging results suggest future development of using the FastFung medium in clinical
31 mycology and in mycobiome characterization. Further prospective evaluation aiming at
32 assessing whether implementing the FastFung medium in the routine workflow simplifies and
33 strengthen fungal isolation capacities in the clinical laboratory is warranted.

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35

36

37 **Keywords:** culture media; clinical laboratory techniques; mycology; mycoses; Malassezia;
38 *Candida*; *Cryptococcus*; mycobiome.

39

40

41 INTRODUCTION

42 Fungal species are ubiquitous environmental organisms. They can be found in food, water, soil
43 and also isolated from humans and animals samples (1). They are often associated with some
44 human infectious diseases (2;3). Fungal microorganisms of clinical interest are mainly grouped
45 into three classes: Ascomycota, Basidiomycota and Zygomycota (4). Fungal infections are the
46 current object of many studies (1;2;5). The predominant clinical species is *Candida albicans* that
47 is involved in the majority of opportunistic invasive fungal diseases (6). Indeed, recent studies
48 have pointed out *C. albicans* as a major cause of morbidity and mortality in critical care patients,
49 as well as its involvement in other oropharyngeal diseases of the mouth, and digestive and
50 vaginal tract in immunocompromised and antibiotic treated individuals (7). The genera
51 *Cryptococcus* and *Aspergillus* also include many fungi involved in human infections (2;8). Many
52 fungal species are opportunistic pathogens; *i.e.* they are commensals when the host is healthy,
53 and they become pathogenic when the host is immunocompromised and/or critically ill (7).
54 Thus, the population of immunocompromised patients has the highest risk of opportunistic
55 fungal infections (2;3;9). Moreover, recent increases in emerging fungal infections call for an
56 effective culture medium capable of timely isolating fastidious fungi from any biological sample
57 at the clinical mycology laboratory.

58 While Sabouraud dextrose agar is the most frequently used medium for fungal isolation (10;11),
59 other media are commonly used for culture of yeasts and filamentous fungi, including Potatoe
60 Dextrose Agar (12), Czapek (13) and Dixon agar (14). These media are often used in
61 combination in clinical laboratories. These, and several other culture media, are used as reference
62 for the isolation or the identification of specific fungal taxa (15). This study aimed at developing
63 a novel culture medium, which is suitable for the culture of clinical fungi, including fastidious
64 ones, for both research and diagnostic studies. The FastFung medium is based on Schædler agar
65 (Sigma-Aldrich) supplemented with many components needed for the growth of fungal species.

66 It also contains selective antibacterial agents for the inhibition of contaminant bacteria. In this
67 preliminary study, the FastFung medium was evaluated on 98 fungal and 20 bacterial strains.

68

69 **MATERIALS AND METHODS**

70 **Culture Media**

71 In this study the performance of fungal culture on FastFung and Sabouraud dextrose agar
72 (Oxoid, Dardilly, France) media were compared. The Sabouraud medium is supplemented with
73 chloramphenicol and gentamycin. In addition to bacteriological agar, this culture medium
74 contains glucose and peptone allowing the growth of many fungal species. Sabouraud medium
75 was considered as the gold-standard fungal medium in this study. The FastFung medium
76 proposed in this study is composed per liter of 43 g of Schædler Agar (Sigma-Aldrich, Saint-
77 Quentin Fallavier, France), 20 g of peptone and 10 g of glucose as basic components, with the
78 addition of 5 g of ox-bile 10 g of malt extract, 2 ml of oleic acid, 5 ml of Tween 60 and 2.5 ml of
79 glycerol. Each component was autoclaved at 121°C for 30 min; except for Tween 60, glycerol
80 and oleic acid, which were added after autoclaving. After cooling to 50°C, the FastFung medium
81 was supplemented with a mixture of three antibiotics at 30 mg/l, including colistin, vancomycin
82 and imipenem (Sigma Aldrich, St Louis, France). The final product was then dispensed into 90
83 mm sterile Petri dishes.

84 **Bacterial and fungal strains**

85 A collection of 98 fungal strains (Table 1) and 20 bacterial strains (Table 2) that were isolated in
86 the laboratory by culturomics methods (16;17) were used to assess the FastFung medium. The
87 identification of each strain was confirmed by the sequence analysis of the partial 16S rRNA
88 gene for bacteria or the ITS rRNA region for fungi, as previously described (16;18). The fungal

89 strains used included *Candida* spp. (n = 50), *Cryptococcus* spp. (n = 2), *Aspergillus* spp. (n = 7),
90 *Penicillium* spp. (n = 3), *Malassezia* spp. (n = 2) and 34 other fungal strains (Table 1).
91 Appropriate negative and positive controls were used in each experiment.

92 **Evaluation of FastFung medium and Sabouraud agar on fungal strains**

93 All the strains used in this study have been preserved at - 80°C in sterile tubes with glycerol. The
94 fungal strains have been cultured onto Sabouraud, Dixon agar or Potatoes Dextrose agar and the
95 bacterial strains on 5% sheep blood-enriched Colombia agar (BioMérieux, France) to obtain
96 fresh and pure cultures. Each isolate was suspended in 1 ml of saline (0.85%) at a concentration
97 of approximately 1.5×10^5 CFU/ml for fungi and 1.5×10^8 CFU/ml for bacteria (0.5 McFarland
98 standard) using a densitometer (DEN-1 McFarland Densitometer, BioSan, Riga, Latvia). Serial
99 five-fold inoculum dilutions were then prepared as follows: 100 µl of each solution was diluted
100 in 900 µl of water following by four other serial dilutions. A 1 µl aliquot of each dilution was
101 inoculated onto the culture plate and spread with a sterile inoculation loop. All plates were
102 incubated at 28°C and 32°C for fungi and 37°C for bacterial strains under aerobic conditions.
103 The bacterial strains were inoculated in the same way as fungal strains, but no dilution was done.

104 **Assessment of the FastFung medium on routine clinical samples**

105 The effectiveness of the FastFung medium was further assessed on 120 clinical samples,
106 prospectively received at the Timone University Hospital (Marseille, France) microbiology
107 laboratory for routine analyses for a 6 week period in which a fungus was isolated. These
108 samples were obtained from various body sites, mostly urine and vagina, as detailed in Table 3.
109 An aliquot (50 µl) of each clinical sample was inoculated onto the Sabouraud and the FastFung
110 media in parallel. The plates were incubated under aerobic conditions from 24 to 48 hours at
111 28°C.

112 **FastFung medium stability assessment**

113 Forty FastFung medium plates were prepared and stored at 4°C for 4 weeks. Six fungal strains
114 including *Candida* spp. (n = 2), *Cryptococcus* spp. (n = 2) and *Penicillium* spp. (n = 2), were
115 used to test the stability of the culture medium. The same procedures described above were
116 applied to inoculate fungal strains onto the FastFung medium. Inoculated plates were then
117 incubated under aerobic conditions at 28°C and examined daily for 7 days.

118

119 **RESULTS**

120 **FastFung medium assessment on fungal strains**

121 The positive culture rate of the fungal strains after 6 days 98/98 (100%) with the FastFung
122 medium. Moreover, each inoculum dilution yielded a positive culture with the FastFung
123 medium. Among each fungal strain assayed on the FastFung medium, growth was visualized in
124 57/98 (58%) of the cultures after 24 hour of incubation and in 67/98 (68%) after 48 h of
125 incubation. In contrast, the Sabouraud medium allowed the growth of 93/98 (95%) of the fungal
126 strains, and in particular, *Candida zeylanoides*, *Cryptococcus diffluens*, *Cryptococcus*
127 *uniguttulatus*, *Malassezia furfur* and *Torulaspora pretoriensis* did not grow (Table 1). Among
128 bacterial strains inoculated onto the FastFung medium plates, 20/20 (100%) bacterial strains
129 were effectively inhibited and did not grew on the FastFung medium (Table 2). In contrast, only
130 4/20 (20%) of the bacterial strains were inhibited and did not grow on the Sabouraud medium
131 (Table 2).

132

133 **FastFung medium assessment on prospective routine clinical samples**

134 There was no discrepancy between the two media regarding the positive culture rate. All the
135 tested were culture positive samples grew on both Sabouraud and FastFung culture media (Table

136 3). *Candida albicans*, *Candida tropicalis* and *Candida parapsilosis* were the most frequently
137 isolated fungal species. *Candida albicans* was the most frequent isolated species, regardless of
138 the clinical sample type and the culture medium. *Candida albicans* and *Candida tropicalis* were
139 relatively more frequently isolated from urinary samples. *Candida tropicalis* and *Candida*
140 *parapsilosis* were relatively more frequently isolated from bronchial aspiration samples. After
141 only 24 hours of incubation the number of fungal CFU was higher on the FastFung medium than
142 on the Sabouraud medium (Figure 1). Moreover, several filamentous fungi contaminations
143 occurred on Sabouraud but not on FastFung medium (data not shown).

144

145 **FastFung medium stability**

146 Neither the quality of growth of the six fungal strains nor the inhibition bacterial strains and
147 filamentous fungi was altered when the culture medium had been stored at 4°C for 4 weeks (data
148 not shown).

149

150 **DISCUSSION**

151 Overall the FastFung medium proved more efficient than the Sabouraud medium for the rapid
152 isolation of fungi and inhibition of bacteria and filamentous fungi contaminants from the clinical
153 samples tested. Each of the 98 fungal strains tested grew on the FastFung medium, whereas only
154 67 grew on Sabouraud. A number of fastidious fungi, such as the lipid dependent *Malassezia*
155 spp. yeast, require the specific culture media for their growth (14;15). The addition of specific
156 components, such as oleic acid, considerably extended the spectra of culturable fungi as
157 described previously (20). In addition, and although both two culture media contain
158 antibacterials, the FastFung medium more efficiently inhibited bacterial growth than the
159 Sabouraud medium.

160 Studies comparing the efficiency of different fungal culture media are scarce. To our knowledge,
161 we report the first study that compares a new fungal culture medium with a reference culture
162 medium commonly used for the isolation and culture of fungal species. One strengths of this
163 study is the large number of bacterial and fungal strains assessed, which were all identified by
164 MALDI TOF mass spectrometry and DNA sequence analysis (19).

165 When compared to the Sabouraud medium on routine clinical samples, FastFung medium
166 displayed a higher fungal colonies count after 48 hours of culture (Figure1) a lower number of
167 commonly encountered culture contamination (10;21). The FastFung medium proved stable for 4
168 weeks. Using this versatile culture medium in the clinical laboratory would advantageously
169 avoid using various fungal culture media for the isolation of fastidious fungi and offers great
170 opportunities in clinical microbiology and culturomics studies when associated with MALDI
171 TOF mass spectrometry identification based on comprehensive fungal reference spectra
172 databases.

173 In conclusion, implementing the FastFung medium in the routine clinical mycology laboratory
174 workflow would advantageously reduce the time to fungal growth and makes unnecessary the
175 use of Sabouraud and other complementary culture media for fungal isolation. Further
176 prospective evaluation of the use of the FastFung medium for fungal isolation in the clinical
177 laboratory is warranted.

178

179

180

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184

185 **Ethical Approval**

186 Not required

187

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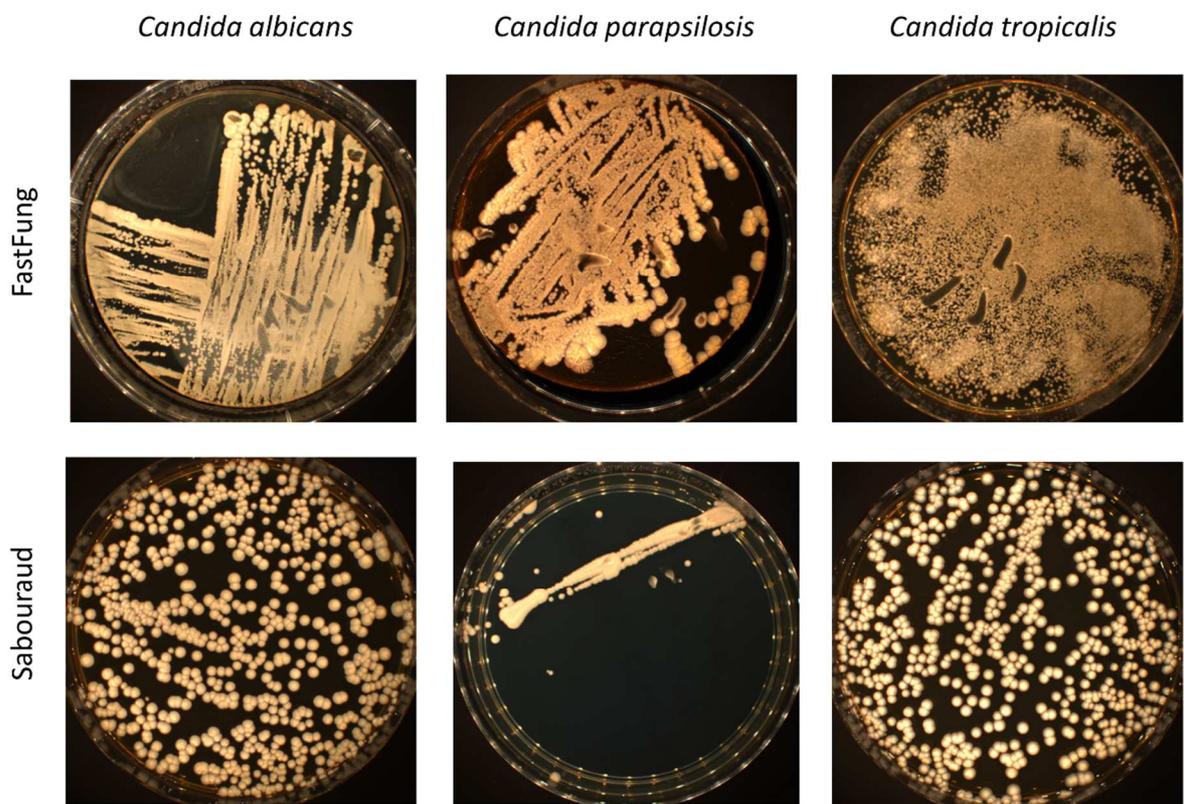
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1
2 **Figure 1.** The FastFung medium displayed higher fungal CFU counts compared to the
3 Sabouraud medium. Here we show the isolation after 48 hours of incubation of three yeast
4 species (*Candida albicans*, *C. parapsilosis*, and *C. tropicalis*) from routine clinical samples that
5 were inoculated in parallel onto both culture media.

6



7

1 **Table 1:** Culture success rates of 98 pure culture clinical fungal isolates inoculated onto
 2 Sabouraud and FastFung media.

3

Fungal species	N	Sabouraud	FastFung
<i>Acremonium kiliense</i>	2	100%	100%
<i>Aspergillus flavus</i>	1	100%	100%
<i>Aspergillus niger</i>	5	100%	100%
<i>Aspergillus sydowii</i>	1	100%	100%
<i>Beauveria bassiana</i>	2	100%	100%
<i>Candida albicans</i>	10	100%	100%
<i>Candida glabrata</i>	6	100%	100%
<i>Candida guilliermondii</i>	5	100%	100%
<i>Candida kefyri</i>	4	100%	100%
<i>Candida lusitanae</i>	3	100%	100%
<i>Candida orthopsilosis</i>	6	100%	100%
<i>Candida parapsilosis</i>	6	100%	100%
<i>Candida tropicalis</i>	6	100%	100%
<i>Candida zeylanoides</i>	1	0%	100%
<i>Candida krusei</i>	3	100%	100%
<i>Clavispora lusitanae</i>	2	100%	100%
<i>Cryptococcus diffluens</i>	1	0%	100%
<i>Cryptococcus unigutulatus</i>	1	0%	100%
<i>Debaryomyces hansenii</i>	2	100%	100%
<i>Galactomyces geotrichum</i>	1	100%	100%
<i>Isaria farinosa</i>	1	100%	100%
<i>Malassezia furfur</i>	1	0%	100%
<i>Malassezia pachydermatis</i>	1	100%	100%
<i>Mucor circinelloides</i>	1	100%	100%
<i>Mucor velutinosus</i>	1	100%	100%
<i>Penicillium chrysogenum</i>	1	100%	100%
<i>Penicillium glandicola</i>	1	100%	100%
<i>Penicillium dipodomyicola</i>	1	100%	100%
<i>Pichia caribbica</i>	3	100%	100%
<i>Pichia manshurica</i>	1	100%	100%
<i>Rhodotorula mucilaginosa</i>	6	100%	100%
<i>Saccharomyces cerevisiae</i>	6	100%	100%
<i>Trichosporon asahii</i>	4	100%	100%
<i>Preussia minima</i>	1	100%	100%
<i>Torulasporea pretoriensis</i>	1	0%	100%
Total	98	95%	100%

4

1 **Table 2:** Isolation rates of pure bacterial strains inoculated onto Sabouraud and FastFung media.

2

Bacterial strains	N	Sabouraud	FastFung
<i>Acinetobacter baumannii</i>	2	100%	0%
<i>Enterobacter cloacae</i>	2	100%	0%
<i>Enterococcus faecalis</i>	2	100%	0%
<i>Escherichia coli</i>	2	100%	0%
<i>Gardnerella vaginalis</i>	2	100%	0%
<i>Haemophilus influenzae</i>	2	100%	0%
<i>Klebsiella oxytoca</i>	2	100%	0%
<i>Klebsiella pneumoniae</i>	2	100%	0%
<i>Proteus mirabilis</i>	1	0%	0%
<i>Pseudomonas aeruginosa</i>	1	0%	0%
<i>Rhizobium radiobacter</i>	1	0%	0%
<i>Staphylococcus aureus</i>	1	0%	0%
Total	20	80%	0%

3

1 **Table 3:** Fungi cultivation rates from various routine clinical samples inoculated onto Sabouraud
 2 and FastFung media. .

Samples	Species	N	Sabouraud	FastFung
Vaginal	<i>Candida albicans</i>	52	100%	100%
Urines	<i>Candida albicans</i>	70	100%	100%
Urines	<i>Candida tropicalis</i>	15	100%	100%
Swab	<i>Candida albicans</i>	4	100%	100%
Nasopharyngeal aspiration	<i>Candida albicans</i>	4	100%	100%
Bronchial aspiration	<i>Candida tropicalis</i>	2	100%	100%
Bronchial aspiration	<i>Candida parapsilosis</i>	3	100%	100%
Sputum	<i>Candida albicans</i>	6	100%	100%
Pulmonary aspiration	<i>Candida albicans</i>	2	100%	100%
Aortic valve	<i>Candida albicans</i>	2	100%	100%
Biopsy	<i>Candida albicans</i>	1	100%	100%
Total		161	100%	100%

3

4